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For: **NOTOGINSENG SAPONIN INTRAVENOUS INJECTION AND THE
METHOD FOR PREPARING THIS INJECTION**

Commissioner for Patents

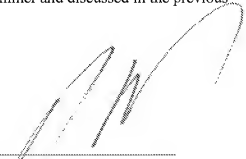
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**SUPPLEMENT TO SUBMISSION ACCOMPANYING REQUEST FOR CONTINUED
EXAMINATION**

Submitted herewith is a translation into English of the whole of the Gai et al Gai et al
Chinese Publication CN 1,273,114 cited by the examiner and discussed in the previous
submission

Respectfully submitted,



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PROCESS FOR REFINING INJECTION "XUESAITONG"

The present invention relates to the field of medical formulation. In particular, the present invention relates to the process for refining the Xuesaitong Injection.

Notoginseng is an *Araliaceous* herbaceous perennial, and the root thereof comprises a variety of saponins. Radix Notoginseng is a traditional Chinese medicine which has functions of expanding blood vessels and inhibiting aggregation of blood platelets. The "Xuesaitong" injection made from the saponin family of Radix Notoginseng as active ingredient has clinical effects of promoting blood circulation, removing blood stasis, unobstructing arteries and activating the veins; and can be useful in the treatment of disorders of cardio- and cerebro-vascular systems, sequelae of cerebrovascular diseases, central retinal vein occlusion, hyphema, and the like.

Currently, the process of producing the powders of saponin family of Radix Notoginseng does not comprise any relatively effective filtering operation, nor does it comprise an effective procedure for depositing impurities. Thus, the produced powders of saponin family of Radix Notoginseng have a relatively lower net concentration of Notoginseng saponin family, and the saponin family of Radix Notoginseng contained in the Xuesaitong injection made from such powders of saponin family of Radix Notoginseng can only reach 85% of the labeled amount (mg/ml). Moreover, there are still some problems present in terms of the drug stability and clarity of the prepared injection.

The object of the present invention is to provide a process of producing Xuesaitong injection having a high level of saponin family of Radix Notoginseng, superior clarity, and good stability.

To achieve the above described object, the inventor utilizes a process of producing the refined Xuesaitong injection comprising the steps of:

- 1) decocting raw herbs of Radix Notoginseng in an appropriate amount of boiling water for 1 to 2 hours and filtering the decocta, followed by decocting additionally the filtered residues in an appropriate amount of boiling water for 1 to 2 hours and filtering the decocta;
- 2) combining and concentrating the filtrates obtained in the first and second decoctions, and then adding ethanol into the concentrated solution until the ethanol level in the solution reaches 75% to 85%;
- 3) allowing the obtained solution to flow through a resin column, and stand under a cold storage condition of 0°C to 5°C for 24 to 48 hours;
- 4) recovering the ethanol from the solution which had been subject to the cold

storage treatment;

- 5) filtering the above obtained solution with a microporous filter membrane, and adjusting the pH to 4.8 to 6.0;
- 6) concentrating and drying the solution obtained in step 5) to produce powders of saponin family of Radix Notoginseng;
- 7) mixing the aforesaid powders of saponin family of Radix Notoginseng with water for injection at a weight ratio of 0.8-1.2:800-1200, and then adjusting the pH to 5.0-7.0;
- 8) boiling the obtained solution for 15 to 20 minutes, and adding 0.25 to 0.5 wt% of activated carbon;
- 9) filtering the obtained mixture, and performing a secondary filtration; and
- 10) packing and sterilizing the filtered solution to produce the desired Xuesaitong injection.

The process of the present invention has the following advantages: the filtration of the solution with a resin column plays an essential role in the substantial reduction of impurities contained in the solution; the use of cold storage (i.e., depositing impurities by cold storage following heat treatment) further deposits impurities during the cooling procedure; the pH adjustment after recovering ethanol produces an active effect for the elimination of impurities. Thus, the prepared solution has a remarkably improved net concentration of the saponin family of Radix Notoginseng, thereby providing a basis of improvement of clarity and stability of the Xuesaitong injection. The Xuesaitong injection prepared according to the process of the present invention represents substantially improved clarity and stability, and the level of saponin family of Radix Notoginseng in the injection can reach up to 90.0 to 110.0% of the labeled amount (mg/ml). That is to say, the level of active ingredients of the Xuesaitong injection prepared according to the process of the present invention is substantially increased.

The animal experiments for testing the acute toxicity of the Xuesaitong injection and the pharmacodynamic study indicate that the injection according to the present invention is an actually safe medicament, which is capable of remarkably inhibiting and antagonizing the formation of thrombus, decreasing permeability of cerebral blood vessels, reducing hydrocephalus, increasing the oxygen-deficit resistance of mice, inhibiting the aggregating function of blood platelets, reducing blood viscosity, changing the rheological property of blood, as well as reducing blood coaggregation and extending the coagulation time. Through statistic treatment, the experimental results of the injection according to the present invention represents substantial differences as compared to the

control group or the model group, thereby indicating that the injection is a good medicament for promoting blood circulation and removing blood stasis. Thus, the injection according to the present invention is an effective medicament for the treatment of disorders of cardio- and cerebro-vascular systems.

Hereinafter the present invention is further described by referring to the examples.

Example 1:

1. 2 kg of Radix Notoginseng raw herbs were boiled and decocted in an appropriate amount of tap water for 2 hours, and then the decocta was filtered. The filtered residues were further boiled and decocted in an appropriate amount of tap water for additional 2 hours, and then the decocta were filtered;
2. The filtrates obtained in the first and the second decoction were combined, heated at 75°C, and concentrated to a specific weight of 1.12. Ethanol (95%) was added into the concentrated solution to a level of 75%, such that the concentrated solution was subjected to a deposition treatment;
3. The solution was filtered with a column of polyamide macroporous resin, and stood under a cold storage condition of 4°C for 40 hours such that the solution was subject to a cold deposition after heat treatment;
4. Recover ethanol from the solution after cold storage by using an ethanol recovery tower;
5. The solution from which ethanol had been removed was filtered with a 0.65 µm microporous filter membrane, and then adjusted to pH ~ 5.5 with HCl (2 mol/L);
6. The obtained solution was heated at 75°C, concentrated to a specific weight of 1.12, and vacuum dried at a negative pressure of 0.15 Pa to produce powders of saponin family of Radix Notoginseng (20 g);
7. 20 g of the above-prepared powders of saponin family of Radix Notoginseng were mixed with 20 kg of water for injection, and adjusted to pH ~ 6.0 with an aqueous NaOH solution (40%);
8. The obtained solution was boiled for 20 minutes, and then 0.4% of activated carbon was added thereto;
9. The mixture was filtered to remove the activated carbon by using filter papers, and then subject to a secondary filtration by using a 0.25 µm microporous filter membrane;
10. The prepared solution was packed into vials and sterilized with UV radiation to produce the desired Xuesaitong injection.

Example 2:

1. 2 kg of Radix Notoginseng raw herbs were boiled and decocted in an appropriate amount of tap water for 1 hour, and then the decocta was filtered. The filtered residues were further boiled and decocted in an appropriate amount of tap water for additional 1 hour, and then the decocta were filtered;
2. The filtrates obtained in the first and the second decoction were combined, heated at 80°C, and concentrated to a specific weight of 1.10. Ethanol (95%) was added into the concentrated solution to a level of 85%, such that the concentrated solution was subjected to a deposition treatment;
3. The solution was filtered with an anion resin column, and stood under a cold storage condition of 0°C for 24 hours such that the solution was subject to a cold deposition after heat treatment;
4. Recover ethanol from the solution after cold storage by using an ethanol recovery tower;
5. The solution from which ethanol had been removed was filtered with a 0.65 µm microporous filter membrane, and then adjusted to pH ~ 4.8 with HCl (2 mol/L);
6. The obtained solution was heated at 80°C, concentrated to a specific weight of 1.10, and vacuum dried at a negative pressure of 0.1 Pa to produce powders of saponin family of Radix Notoginseng (19 g);
7. 8 g of the above-prepared powders of saponin family of Radix Notoginseng were mixed with 12 kg of water for injection, and adjusted to pH ~ 5.0 with an aqueous NaOH solution (40%);
8. The obtained solution was boiled for 20 minutes, and then 0.25% of activated carbon was added thereto;
9. The mixture was filtered to remove the activated carbon by using filter papers, and then subject to a secondary filtration by using a 0.8 µm microporous filter membrane;
10. The prepared solution was packed into vials and sterilized with UV radiation to produce the desired Xuesaitong injection.

Example 3:

1. 2 kg of Radix Notoginseng raw herbs were boiled and decocted in an appropriate amount of tap water for 1.5 hours, and then the decocta was filtered. The filtered residues were further boiled and decocted in an appropriate amount of tap water for additional 1.5 hours, and then the decocta were filtered;

2. The filtrates obtained in the first and the second decoction were combined, heated at 75°C, and concentrated to a specific weight of 1.15. Ethanol (95%) was added into the concentrated solution to a level of 80%, such that the concentrated solution was subjected to a deposition treatment;
3. The solution was filtered with a column of polyamide macroporous resin, and stood under a cold storage condition of 5°C for 48 hours such that the solution was subject to a cold deposition after heat treatment;
4. Recover ethanol from the solution after cold storage by using an ethanol recovery tower;
5. The solution from which ethanol had been removed was filtered with a 0.65 μm microporous filter membrane, and then adjusted to pH ~ 6.0 with HCl (2 mol/L);
6. The obtained solution was heated at 75°C, concentrated to a specific weight of 1.11, and spray dried to produce powders of saponin family of Radix Notoginseng (19.5 g);
7. 10 g of the above-prepared powders of saponin family of Radix Notoginseng were mixed with 15 kg of water for injection, and adjusted to pH ~ 7.0 with an aqueous NaOH solution (40%);
8. The obtained solution was boiled for 20 minutes, and then 0.5% of activated carbon was added thereto;
9. The mixture was filtered to remove the activated carbon by using filter papers, and then subject to a secondary filtration by using a 0.65 μm microporous filter membrane;
10. The prepared solution was packed into vials and sterilized with UV radiation to produce the desired Xuesaitong injection.

We claim:

1. A process of producing a refined Xuesaitong injection characterized by comprising the steps of:

1) decocting raw herbs of Radix Notoginseng in an appropriate amount of boiling water for 1 to 2 hours and filtering the decocta, followed by decocting additionally the filtered residues in an appropriate amount of boiling water for 1 to 2 hours and filtering the decocta;

2) combining and concentrating the filtrates obtained in the first and second decoctions, and then adding ethanol into the concentrated solution until the ethanol level in the solution reaches 75% to 85%;

3) allowing the obtained solution to flow through a resin column, and stand under a cold storage condition of 0°C to 5°C for 24 to 48 hours;

4) recovering the ethanol from the solution which had been subject to the cold storage treatment;

5) filtering the above obtained solution with a microporous filter membrane, and adjusting the pH to 4.8 to 6.0;

6) concentrating and drying the solution obtained in step 5) to produce powders of saponin family of Radix Notoginseng;

7) mixing the aforesaid powders of saponin family of Radix Notoginseng with water for injection at a weight ratio of 0.8-1.2:800-1200, and then adjusting the pH to 5.0-7.0;

8) boiling the obtained solution for 15 to 20 minutes, and adding 0.25 to 0.5 wt% of activated carbon;

9) filtering the obtained mixture, and performing a secondary filtration; and

10) packing the filtered solution into vials, and sterilizing them to produce the desired Xuesaitong injection.

2. The process of producing the refined Xuesaitong injection according to claim 1 characterized in that: in step 2) the solution is heated at a temperature of 70°C to 80°C and concentrated to a specific weight of 1.10 to 1.15.

3. The process of producing the refined Xuesaitong injection according to claim 1 characterized in that: in step 2), the solution has an ethanol concentration of 75% after addition of ethanol.

4. The process of producing the refined Xuesaitong injection according to claim 1 characterized in that: in step 3), the resin column for filtration is a polyamide macroporous resin column.

5. The process of producing the refined Xuesaitong injection according to

claim 1 characterized in that: in step 4), the ethanol is recovered with an ethanol recovery tower.

6. The process of producing the refined Xuesaitong injection according to claim 1 characterized in that: in step 5), the microporous filter membrane as used has a pore size of 0.25 to 0.8 microns.

7. The process of producing the refined Xuesaitong injection according to claim 1 characterized in that: in step 6), the solution is heated at a temperature of 70°C to 80°C and concentrated to a specific weight of 1.10 to 1.12.

8. The process of producing the refined Xuesaitong injection according to claim 1 characterized in that: in step 7), the powders of saponin family of Radix Notoginseng are mixed with water for injection at a weight ratio of 1:1000.

ABSTRACT

The present invention discloses a process of producing a refined Xuesaitong injection, comprising the steps of: 1) decocting raw herbs of Radix Notoginseng twice; 2) combining and concentration the filtrates, and adding ethanol; 3) allowing the solution to flow through a resin column, and standing for cold storage condition; 4) recovering ethanol; 5) filtering the solution with a microporous filter membrane, and adjusting pH; 6) concentrating and drying the solution to produce powders of saponin family of Radix Notoginseng; 7) mixing the powders of saponin family of Radix Notoginseng with water for injection and then adjusting the pH; 8) boiling the solution and adding activated carbon; 9) filtering the obtained mixture, and performing a secondary filtration; and 10) producing the Xuesaitong injection. The Xuesaitong injection prepared according to the process of the present invention represents a high clarity and a long storage period, and can be useful in treatment of cardiovascular diseases.